[CONTRIBUTION FROM THE DEPARTMENT OF RESEARCH IN PURE CHEMISTRY, MELLON INSTITUTE OF INDUSTRIAL RESEARCH]

Cinchona Alkaloids in Pneumonia. IV. Derivatives of Ethylapocupreine[1]

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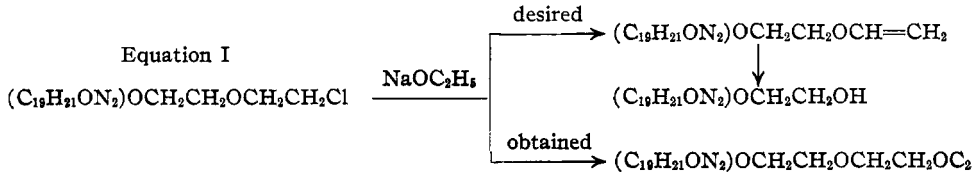
The pneumococcus inhibiting action of various cupreine derivatives and reasons for renewing chemotherapeutic investigations in this field have been given in previous papers of this series.[2,3] In the present paper ethylapocupreine[4] and several of its derivatives are briefly discussed. A preliminary report of the high bacteriostatic activity, high protective action using mice, and lowered toxicity of ethylapocupreine compared to optochin was given at the September, 1934, meeting of the American Chemical Society at Cleveland, Ohio, and discussed in the medical literature[5] by the physicians associated with us. These valuable properties were thoroughly confirmed during the fall and winter of 1934–35. The results are in general agreement with those reported by others[6] although the rather large difference in favor of ethylapocupreine over optochin reported by the Japanese investigators[6b] was not confirmed here. It was later demonstrated by means of a newly developed method using dogs[7] that in large doses this ether as well as optochin had a decidedly damaging effect on the inner ganglionic layer of the retina. Extensive clinical investigation of the drug, therefore, was not carried out. A minor development was the successful use of ethylapocupreine without any unfavorable symptoms in the treatment of pneumococcic infections of the eyes and throat. In this connection the drug is believed to be at least as effective as optochin.

It was believed, on general grounds, and from past experience with hydroxyethylhydrocupreine[8] that the introduction of the hydroxyl group into the ethyl radical of ethylapocupreine would materially decrease its toxicity. The preparation of hydroxyethylapocupreine[8] is not as simple a matter as would at first appear. Ordinary methods, such as alkylation of apocupreine[2,10] in the form of sodium or potassium salt with ethylene chlorohydrin or hydroxyethyl toluenesulfonate yield the desired product but in amounts which are far from satisfactory. Further, the hydroxyethylapocupreine is so contaminated with by-products of uncertain constitution that isolation and purification are tedious and difficult. This result is no doubt due to the presence in the cinchona structure of several reactive groups, other than the phenolic hydroxyl, which is the only point we desire to attack; and to the rather high reactivity of these hydroxyethylating reagents. Various indirect methods such as alkylation of apocupreine N-oxides, and alkylations with toluenesulfonyl esters of glycol monocarboxy esters, either failed completely or gave results so low as to make them impractical.

Equation I



A further attempt consisted in the removal of hydrogen chloride from β-chlorethoxyethylapocupreine with the aim of preparing the vinoxyethyl derivative[2] which should hydrolyze readily to give the desired hydroxyethylapocupreine. However, the only substance which could be isolated under our conditions proved to be the carbitol ether of apocupreine. Equation I shows the desired course of reaction and the one actually obtained.

(1) Presented before the Medicinal Chemistry Section at the September, 1936, meeting, of the American Chemical Society, Pittsburgh, Pa.

(2) Butler and Cretcher, THIS JOURNAL, **57**, 1083 (1935).

(3) Butler, Nelson, Renfrew and Cretcher, *ibid.*, **57**, 575 (1935).

(4) We have had no desire to ignore the criticisms of the term "apocupreine" made by Henry and Solomon, *Chemistry and Industry*, **54**, 641 (1935). We still believe the name "apoquinine" to be unsuitable for the purified substances isolated from this crude reaction product. In order to avoid further confusion, however, we shall continue to apply the term "apocupreine" only to the purified base of $[\alpha]_D$ −215°, until we have the opportunity of going further into the matter of the uniformity of Suszko's base [*Rec. trav. chim.*, **52**, 839 (1933)]. Such opportunity has been denied us up to the present time by the press of more practical matters.

(5) Maclachlan, Permar, Johnston and Kenny, *Am. J. Med. Sci.*, **188**, 699 (1934).

(6) (a) Miura and Okamoto, *Japan J. Med. Sci. IV, Pharmacol.*, **5**, 1 (1930); (b) Ishizaka, Okamoto, Miura and Shako, *ibid.*, **7**, 42, 45 (1933); (c) Matsuda, *ibid.*, **8**, 30 (1934); (d) Gundel and Seitz, *Z. Immunitats.*, **80**, 240 (1933); (e) Leibetruth, *ibid.*, **84**, 445 (1935).

(7) Dawson, Permar, Johnston and Maclachlan, *Am. J. Med. Sci.*, in press.

(8) Protected by U. S. and foreign patents.

TABLE I

ANTIPNEUMOCOCCIC ACTIVITY AND PHARMACOLOGICAL PROPERTIES OF ETHYLAPOCUPREINE DERIVATIVES

Drug	*In vitro* inhibits growth in concn. of	Toxicity (20 g. mice). 3 mg.	4 mg.	5 mg.	Deaths at dosages of 6 mg.	7 mg.	8 mg.	Protection 20 g. mice. Survivals at dosage of 3 mg.	Visual disturbance (dogs)
Optochin	1:800,000	2/30	19/30	30/30	...	...	...	23/30	Positive
Ethylapocupreine	1:800,000	1/30	5/30	22/30	28/30	...	...	23/30	Positive
Hydroxyethylapocupreine	1:400,000	..	...	0/10	1/30	7/30	25/30	21/30	Negative
Butoxyethylapocupreine	1:200,000	..	...	...	25/30	15/15	...	...	Negative
Phenoxyethylapocupreine	1:300,000	2/5	20/20	15/15	...	...	...	...	.....

This reaction product proved to have moderate bacteriostatic and protective activity against pneumococcus in mice.

Other special methods of preparing hydroxyethylapocupreine, which we hope may be considerably more useful, are being investigated at present.

As shown in Table I, the expected lowering of toxicity in hydroxyethylapocupreine was accomplished. The bacteriostatic activity of the drug as compared with ethylapocupreine also was diminished considerably. However, the protecting effect when tested against pneumococcus infected mice was maintained at a high level. A further finding of great importance to these investigations was the absence of any unfavorable eye effect in tests by the dog method referred to above.[7] The data shown in Table I as well as results of extensive clinical tests will be reported in detail elsewhere by the medical staff in charge of this phase of the work.

A study of various other alkoxy- and aryloxyethylapocupreines is also in progress. Judging by the examples herein reported, this type of alkylation takes place quite readily on reaction of apocupreine in alkaline alcoholic solution with the desired R-oxyethyltoluenesulfonate. The preparations were undertaken with the two-fold aim of determining their usefulness as pneumococcicidal agents and of investigating their possibilities as intermediates in the preparation of the hydroxyethyl ether.

Butoxyethylapocupreine, as shown in Table I, had moderate activity as a bacteriostatic agent against the pneumococcus. It was rather low in toxicity and showed no eye effect in a small number of trials when tested by the method mentioned above.[7] Phenoxyethylapocupreine also had moderately high bacteriostatic activity. It was, however, more toxic than any cinchona derivative thus far examined. Preliminary attempts at partial hydrolysis of these substances to hydroxyethylapocupreine according to the equation

$$(C_{19}H_{21}ON_2)OCH_2CH_2OR' \longrightarrow (C_{19}H_{21}ON_2)OCH_2\text{-}CH_2OH + R'OH$$

resulted in a complete breakdown of the ethers to apocupreine and presumably glycol and the corresponding alcohol or phenol. Other derivatives which may show a greater difference in stability of ether linkages are also being investigated and will be reported as soon as possible.

Experimental

Ethylapocupreine.—This ether was prepared by alkylation with ethyl *p*-toluenesulfonate or ethyl sulfate, $[\alpha]_D$ −198°.[9] Dihydrochloride from alcohol: $[\alpha]_D$ −237°.[10] *Anal.* Calcd. for $C_{21}H_{26}O_2N_2 \cdot 2HCl$: Cl, 17.2. Found: Cl, 16.8.

Hydroxyethylapocupreine.—Apocupreine in the form of its potassium salt was alkylated in alcoholic solution with one equivalent of ethylene chlorohydrin. The reaction product was worked up as dihydrochloride. On recrystallization from absolute alcohol containing a little ether a pure salt with $[\alpha]_D$ −229° was obtained. The base was recovered from this salt in amorphous condition, $[\alpha]_D$ −194°.

Anal. Base. Calcd. for $C_{21}H_{26}O_3N_2$: C, 71.1; H, 7.4. Found: C, 70.8, 70.6; H, 7.1. *Dihydrochloride.* Calcd. for $C_{21}H_{26}O_3N_2 \cdot 2HCl$: Cl, 16.6; N, 6.6. Found: Cl, 16.2; N, 6.3.

The demonstration of two hydroxyl groups in this substance was accomplished as follows. Two grams of the base was acetylated thoroughly by refluxing with 10 cc. of acetyl chloride for three hours; the base was recovered and again treated with acetyl chloride for two hours. The final amorphous base had $[\alpha]_D$ −51° in absolute alcohol.

Anal. Calcd. for $2CH_3CO$ in $C_{25}H_{30}O_5N_2$: CH_3CO, 19.6. Found: CH_3CO, 20.3, 19.0, 19.1.

β-Chloroethoxyethyl-*p*-toluenesulfonate.—One hundred and twenty-four grams (1 mole) of diethyleneglycol chlorohydrin was heated with 143 g. (0.75 mole) of *p*-toluenesulfonyl chloride in an oil-bath at 142° for ten hours. The product was diluted with benzene and the solution was washed with sodium hydroxide solution. After drying,

(9) $l = 1$; $c = 1$, for all specific rotations herein reported; bases in absolute alcohol, salts in water.

(10) Compare Henry and Solomon, *J. Chem. Soc.*, 1923 (1934).

the benzene was removed under reduced pressure. The yield of oily ester was 130 g. This product proved to be a satisfactory alkylating reagent without any further purification, even though a small sample distilled from a Hickman vacuum still gave low figures when analyzed for sulfur.

Anal. Calcd. for $C_{11}H_{15}O_5SCl$: S, 10.9. Found: S, 9.4, 9.6.

Butoxyethyl-*p*-toluenesulfonate.—This ester was prepared in 74% yield from ethylene glycol monobutyl ether and *p*-toluenesulfonyl chloride in the presence of pyridine by the method which was used in earlier work, for the preparation of ethoxyethyltoluenesulfonate.[2] The product was a heavy oil which could not be distilled readily. It was therefore analyzed after thorough washing and drying.

Anal. Calcd. for $C_{13}H_{20}O_4S$: S, 11.7. Found: S, 11.5.

Phenoxyethyl-*p*-toluenesulfonate.—This reagent was prepared similarly from ethylene glycol monophenyl ether and *p*-toluenesulfonyl chloride by the method described in the earlier paper.[2] The reaction mixture solidified on standing for a short time to a semi-solid crystalline mass which was separated by filtration into a solid and a liquid fraction. The solid material after several recrystallizations from alcohol was obtained in 42.5% yield with melting point 75°.

Anal. Calcd. for $C_{15}H_{16}O_4S$: S, 11.0. Found: S, 10.6.

The rather low yield is due to the fact that *p*-toluenesulfonyl chloride in this case acts, in part, as a chlorinating agent. This was shown by the isolation from the liquid fraction of the reaction product of a 30% yield of β-chloroethyl phenyl ether, m. p. 28°, b. p. 220° (740 mm.). These figures agree with the data given in the literature[11] for this compound.

β-Chloroethoxyethylapocupreine.—Twenty-five grams of apocupreine in form of potassium salt was refluxed in alcoholic solution with 21.7 g. of β-chloroethoxyethyl-*p*-toluenesulfonate for four and one-half hours. The product was worked up in the ordinary way and finally isolated as dihydrochloride; yield 12.5 g. of salt recrystallized from a mixture of alcohol and ether; $[\alpha]D$ −195°.

Anal. Calcd. for $C_{23}H_{29}O_3N_2Cl \cdot 2HCl$: Total Cl, 21.7; Cl ion, 14.5. Found: Total Cl, 21.6; Cl ion, 14.4.

(11) Bently, Haworth and Perkin, *J. Chem. Soc.*, **69**, 165 (1896).

Apocupreine Carbitol Ether.—This substance resulted from an attempt to prepare vinoxyethylapocupreine as follows: 4.9 g. (0.01 mole) of β-chloroethoxyethylapocupreine dihydrochloride was added to 30 cc. of absolute alcohol in which 0.75 g. (0.032 mole) of sodium had been dissolved. Sodium chloride was filtered off and the clear solution was heated in a sealed tube for eight hours at 96°. After cooling, a nearly quantitative yield of sodium chloride was filtered off and the alcohol was removed by distillation under reduced pressure. On warming the residue in dilute hydrochloric acid solution for about fifteen minutes, no odor of acetaldehyde could be detected. The product was then worked up as dihydrochloride. Since it could not be obtained crystalline, the salt was purified by several precipitations from alcoholic solution with ether; yield 3.7 g.; $[\alpha]D$ −183°.

Anal. Calcd. for $C_{25}H_{34}O_4N_2 \cdot 2HCl$: Cl, 14.2; N, 5.6. Found: Cl, 14.3; N, 5.5.

Butoxyethylapocupreine.—Sixty-two grams of apocupreine, alkylated in the usual way with 54.4 g. of butoxyethyl-*p*-toluenesulfonate yielded 38 g. of crude product. Neither the crude base nor the hydrochlorides could be obtained in crystalline condition. The dihydrochloride was therefore partially purified by treating its tepid aqueous solution with nuchar, concentrating to dryness at reduced pressure, and precipitating several times from alcoholic solution with ether $[\alpha]D$ −198°.

Anal. Calcd. for $C_{25}H_{36}O_3N_2Cl_2$: N, 5.8; Cl, 14.7. Found: N, 5.6; Cl, 14.7.

Phenoxyethylapocupreine.—Sixty-two grams of apocupreine, alkylated with 58.4 g. of phenoxyethyl-*p*-toluenesulfonate, yielded 24 g. of base crystallized from alcohol. A small sample of recrystallized material gave $[\alpha]D$ −159°, m. p. 178°. The base gave a difficultly soluble dihydrochloride when treated with a slight excess of aqueous hydrochloric acid.

Anal. Base. Calcd. for $C_{27}H_{30}O_3N_2$: N, 6.5. Found: N, 6.4. *Dihydrochloride.* Calcd. for $C_{27}H_{30}O_3N_2 \cdot 2HCl$: Cl, 14.4. Found: Cl, 13.8.

Summary

Ethylapocupreine and several of its derivatives have been prepared. Results of pharmacological tests of importance in chemotherapeutic studies of pneumonia have been presented briefly.

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